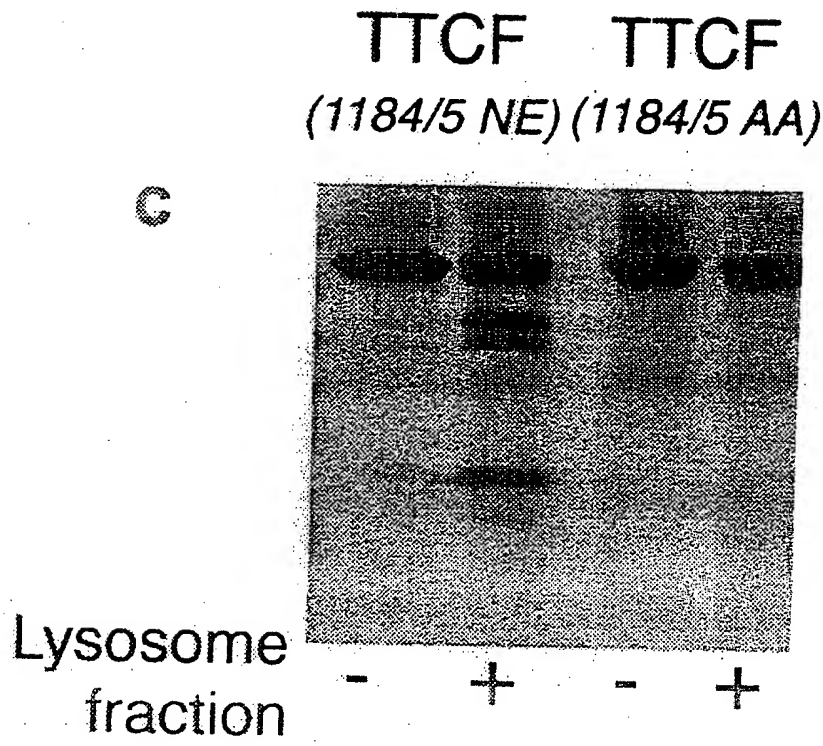
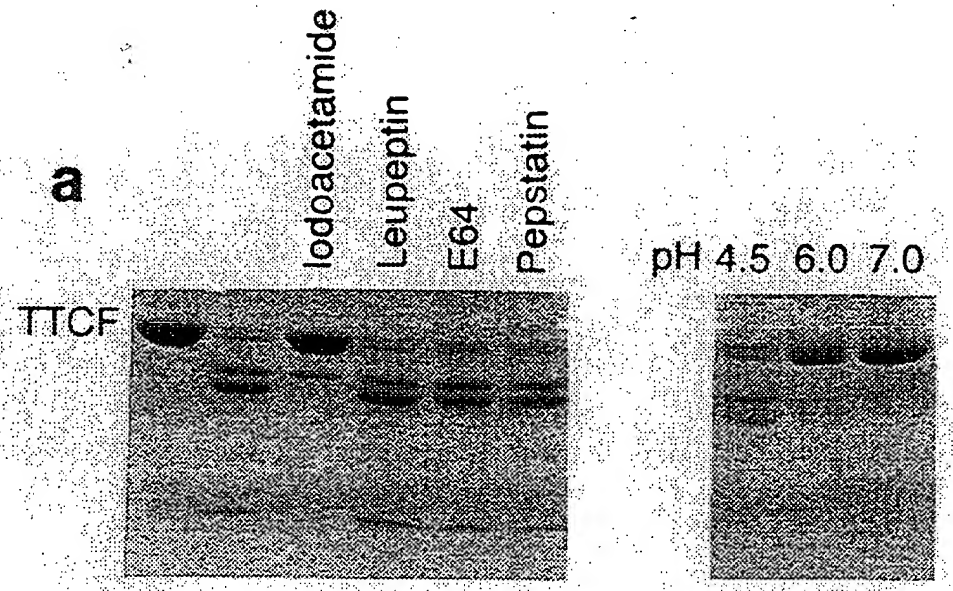


Fig. 1 (page 2 of 2)

Fig 1 (page 1 of 2)



Fragment	Sequence/ cleavage site
2,4	RHIDN **EEDID**
5	YTPNN **EIDSF**
6	GNAFN **NLDRI**

a

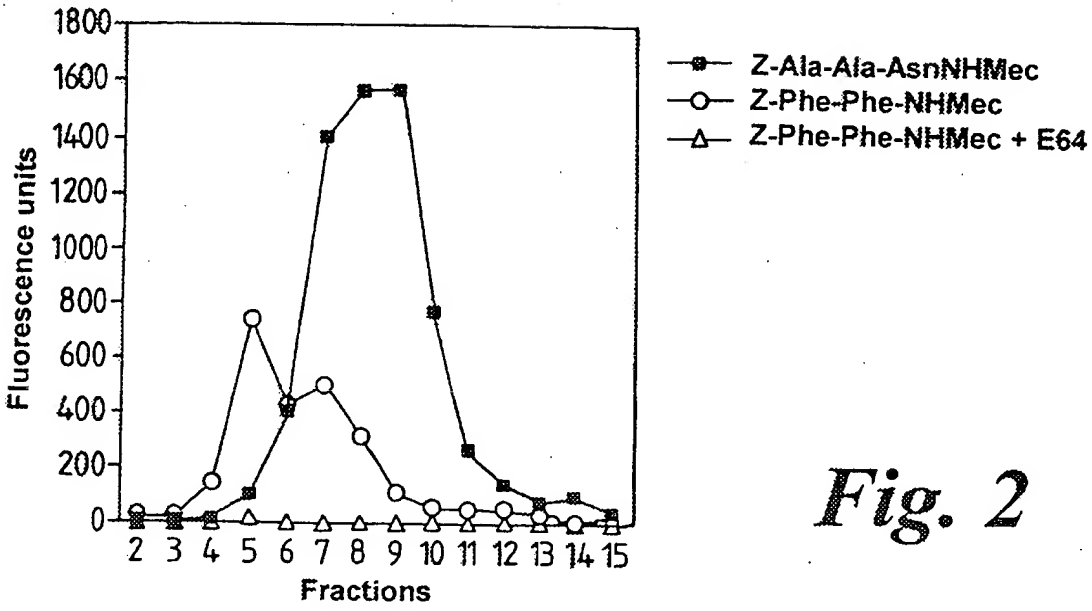
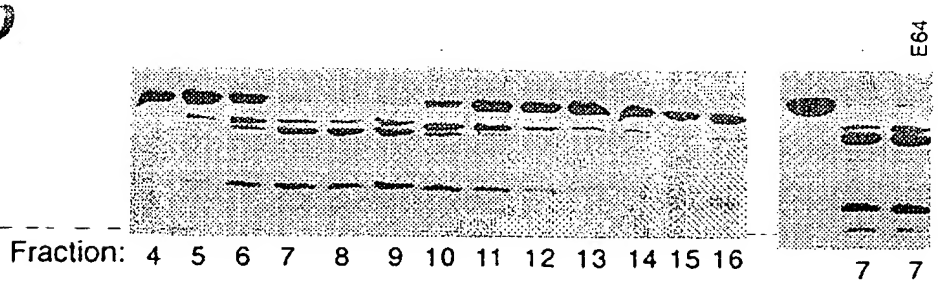
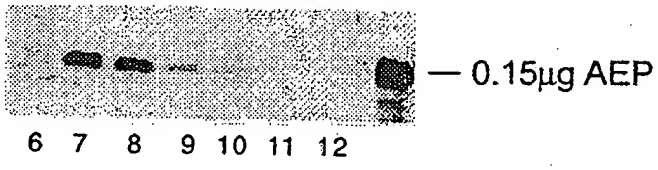

1800
1600
1400
1200
1000
800
600
400
200
0
Fluorescence units
2 3 4 5 6 7 8 9 10 11 12 13 14 15
Fractions
Z-Ala-Ala-AsnNHMec
Z-Phe-Phe-NHMec
Z-Phe-Phe-NHMec + E64

Fig. 2

b


E64
Fraction: 4 5 6 7 8 9 10 11 12 13 14 15 16
7 7

c


— 0.15µg AEP
6 7 8 9 10 11 12

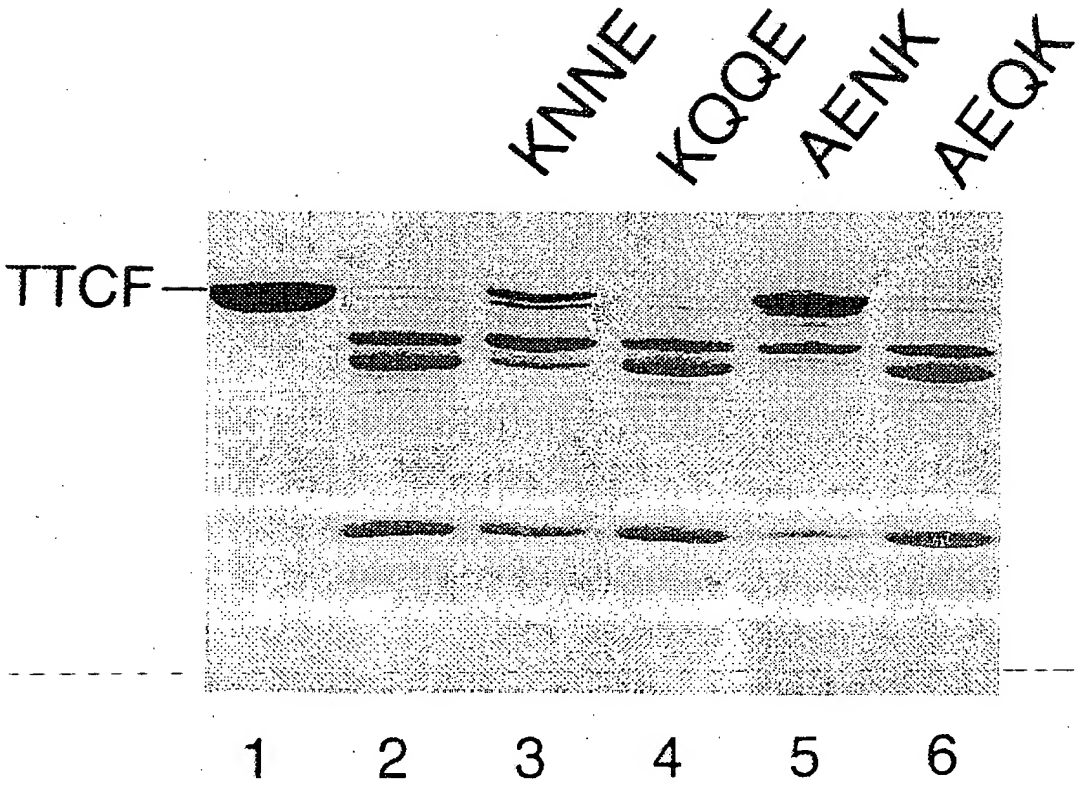


Fig. 3a

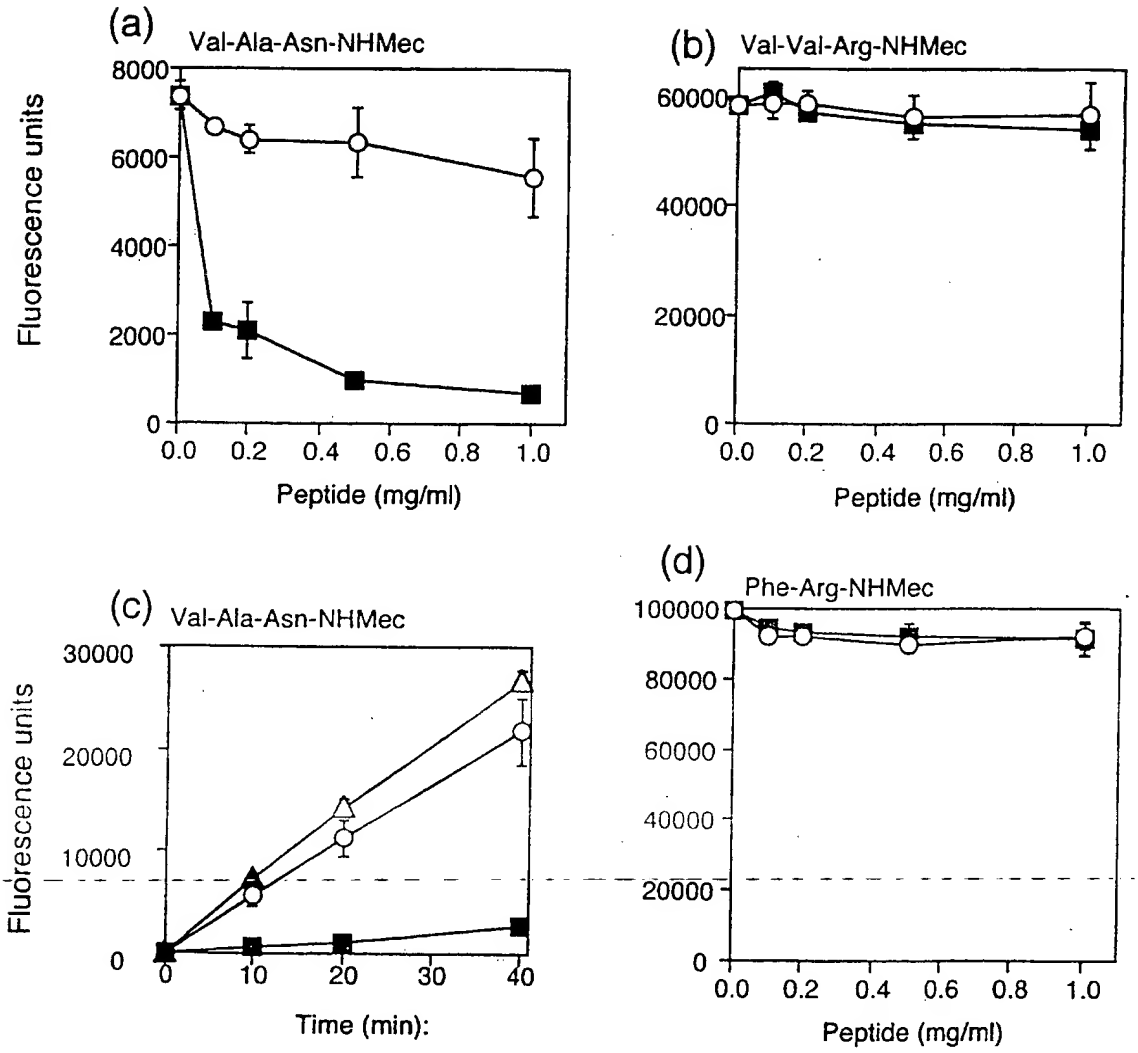

(a)
Val-Ala-Asn-NHMec
Fluorescence units
8000
6000
4000
2000
0
0.0
0.2
0.4
0.6
0.8
1.0
Peptide (mg/ml)
(b)
Val-Val-Arg-NHMec
60000
40000
20000
0
0.0
0.2
0.4
0.6
0.8
1.0
Peptide (mg/ml)
(c)
Val-Ala-Asn-NHMec
Fluorescence units
30000
20000
10000
0
0
10
20
30
40
Time (min):
(d)
Phe-Arg-NHMec
100000
80000
60000
40000
20000
0
0.0
0.2
0.4
0.6
0.8
1.0
Peptide (mg/ml)

Fig. 3b

Fig. 3c

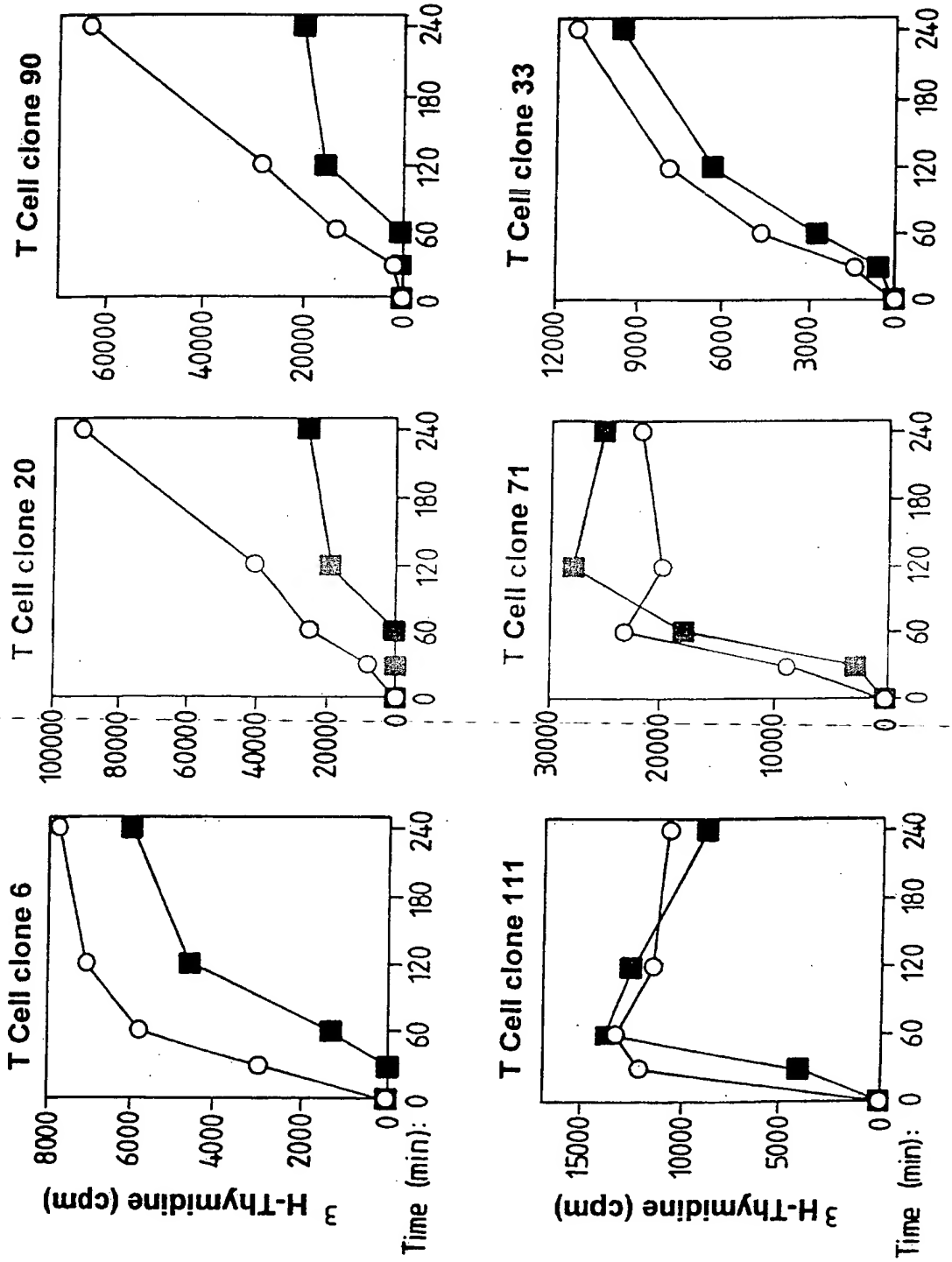

T Cell clone 90
T Cell clone 33
T Cell clone 20
T Cell clone 71
T Cell clone 6
T Cell clone 111
3 H-Thymidine (cpm)
Time (min): 0 60 120 180 240

Fig. 3d

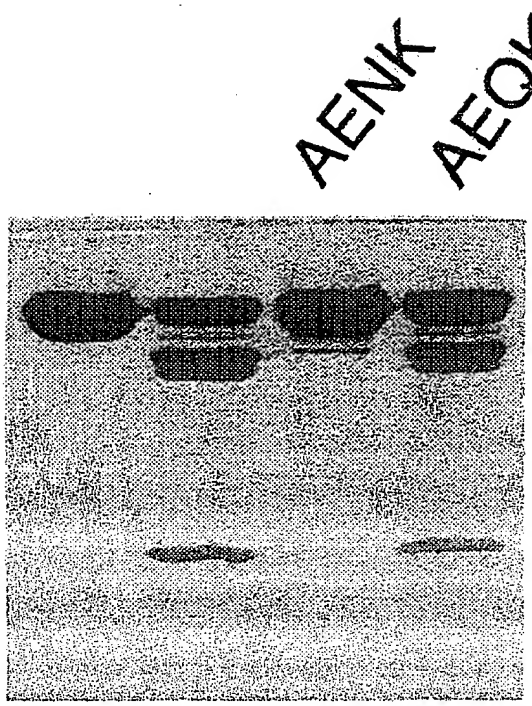
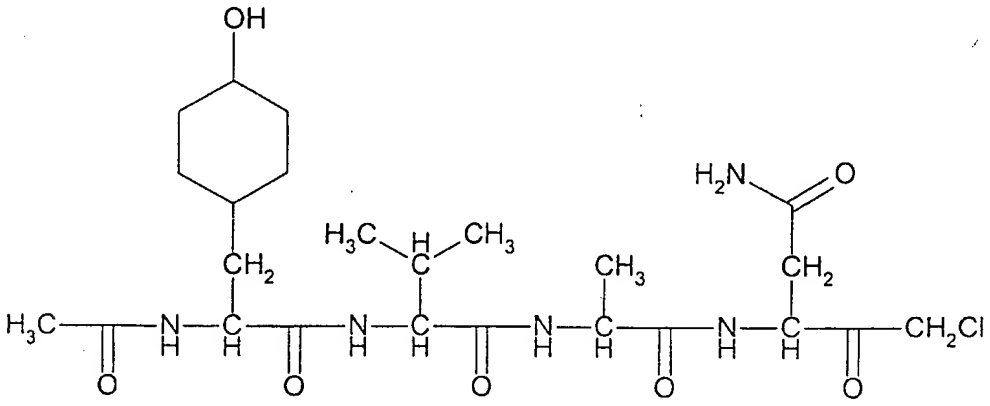


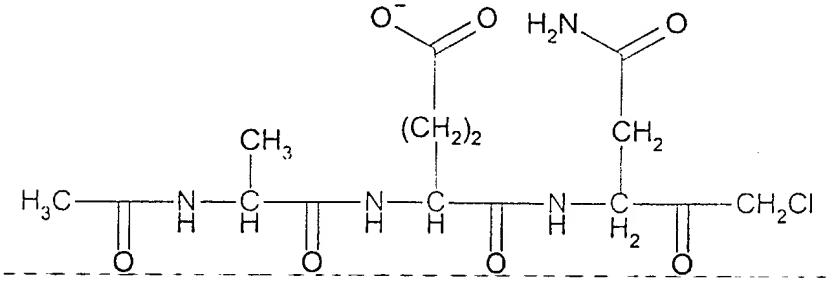
Figure 4 (page 1 of 6)

Peptidyl chloromethylketones (ref 5)

(i) Acetyl-tyrosyl-valyl-alanyl-asparaginyl-chloromethylketone
(analogous to ICE protease inhibitor YVAD-cmk)



(ii) Acetyl-alanyl-glutamyl-asparaginyl-chloromethylketone



(iii) Acetyl (or benzyloxycarbonyl)-$(X)_n$-asparaginyl-chloromethylketone
Where X = any amino acid

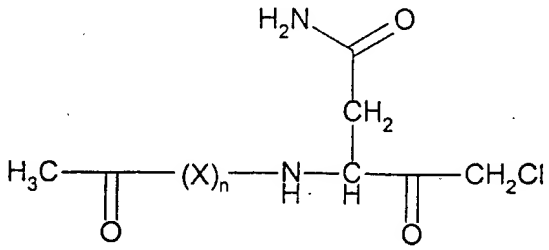
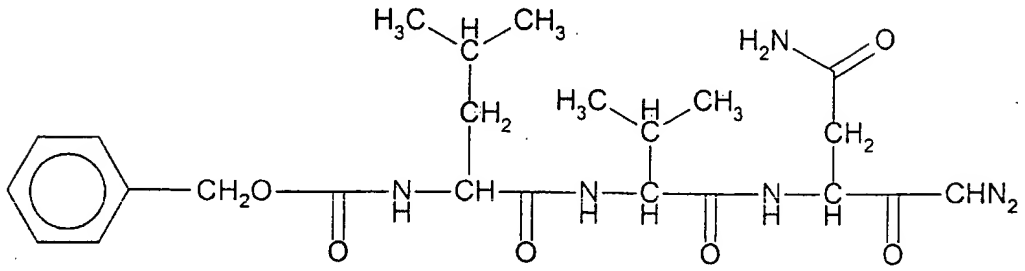


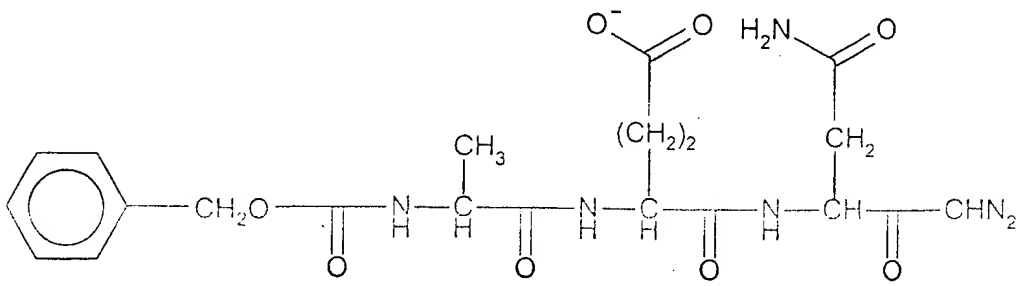
Figure 4 (page 2 of 6)

Peptidyl diazomethanes (ref 3,4)
(have the general structure: $R\text{-}C(=O)CHN_2$)

(i) Benzyloxycarbonyl-leucyl-valyl-asparaginyl-diazomethane



(ii) Alanyl-glutamyl-asparaginyl-diazomethane



(iii) $Z\text{-}(X)_n$-asparaginyl-diazomethane

Where Bl = acetyl or benzyloxycarbonyl and X = any amino acid

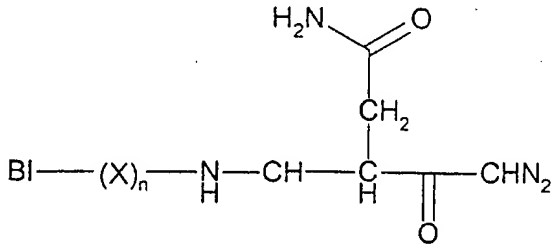
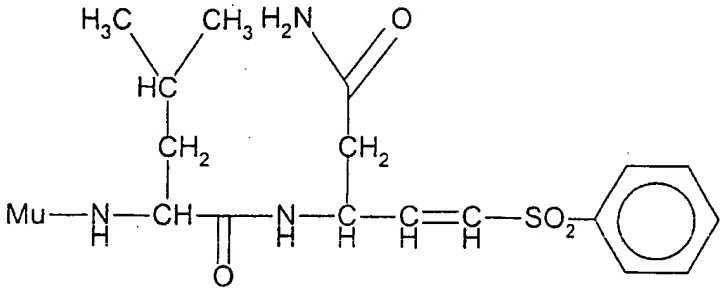


Figure 4 (page 3 of 6)

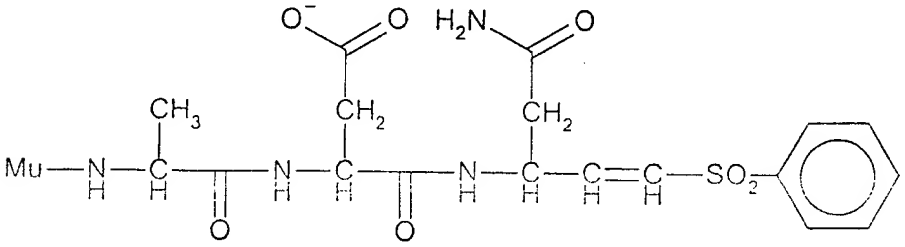
Peptidyl vinyl sulphones (ref 6)

(i) Morpholinurea-leucyl-asparaginyl-vinylsulphone-phenyl
(Acetyl or benzyloxycarbonyl can replace morpholinurea)



Mu - morpholinurea

(ii) Morpholinurea-alanyl-glutamyl-asparaginyl-vinylsulphone-phenyl
(Acetyl or benzyloxycarbonyl can replace morpholinurea)



(iii) Bl-$(X)_n$-asparaginyl-vinylsulphone-R

Where Bl = N-terminal blocking group (acetyl, morpholinurea or benzyloxycarbonyl), X = any amino acid
and R = alkyl or aryl terminating group

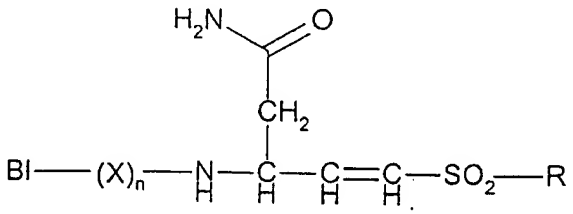
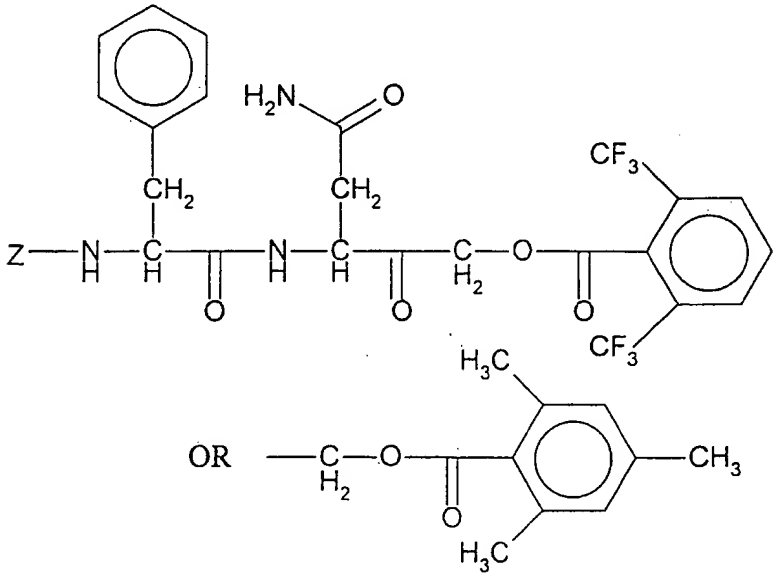


Figure 4 (page 4 of 6)

Peptidyl (acyloxy) methanes (ref 7)

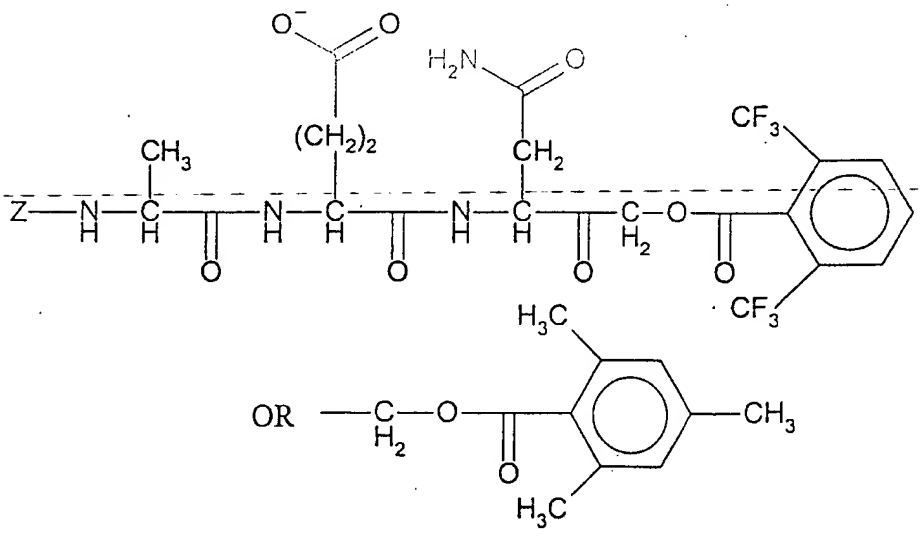
(i) Z-Phenylalanyl-asparaginyl-CH_2OCO-[2,6-$(CF_3)_2$Phenyl]

(ii) Z-Phenylalanyl-asparaginyl-CH_2OCO-[2,4,6-$(CH_3)_3$ Phenyl]



(iii) Z-alanyl-glutamyl-asparaginyl-CH_2OCO-[2,6-$(CF_3)_2$Phenyl]

(iv) Z-alanyl-glutamyl-asparaginyl-CH_2OCO-[2,4,6-$(CH_3)_3$ Phenyl]



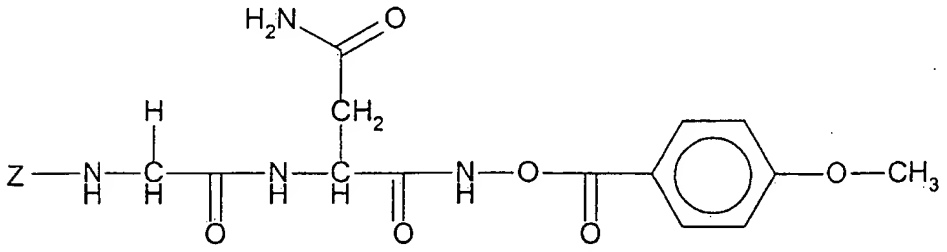
(v) Z-$(X)_n$-asparaginyl-CH_2OCO-R

Where X = any amino acid and R = [2,6-$(CF_3)_2$Phenyl] or [2,4,6-$(CH_3)_3$ Phenyl] or other acyloxy methane group

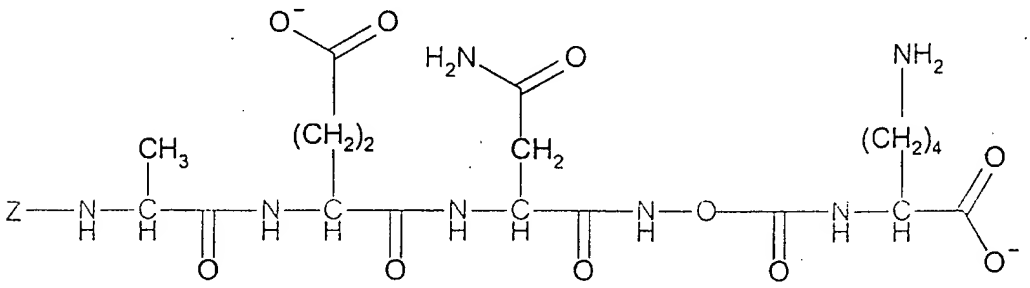
Figure 4 (page 5 of 6)

N,O-diacyl hydroxamates (ref 8)

(i) Z-Glycyl-asparaginyl-NHO-benzoyl(4-OCH_3)



(ii) Z-alanyl-glutamyl-asparaginyl-NHO-CO-lysine-NH



(iv) Z-$(X)_n$-asparaginyl-NHO-CO-R

Where Z = benzyloxycarbonyl or other blocking group,
X = any amino acid and R = any O-acyl group

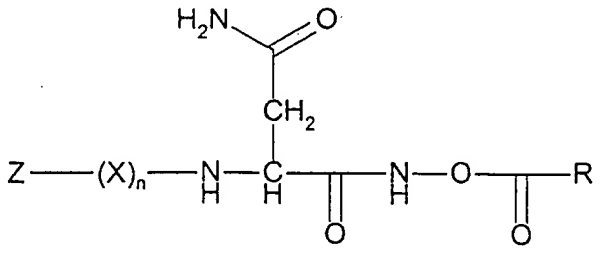
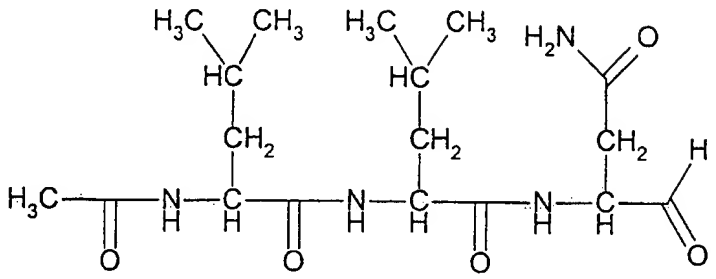


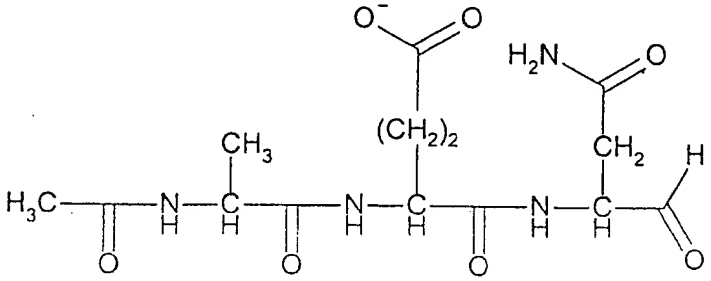
Figure 4 (page 6 of 6)

Peptide aldehydes (refs 1 & 2)

(i) Acetyl-leucyl-leucyl-asparaginal



(ii) Acetyl-alanyl-glutamyl-asparaginal



(iv) Acetyl (or other blocking group)-$(X)_n$-Asparaginal

-where X denotes any amino acid(s) in peptide linkage

Elastinal also blocks AEP. A more specific variant would be:

(iv)

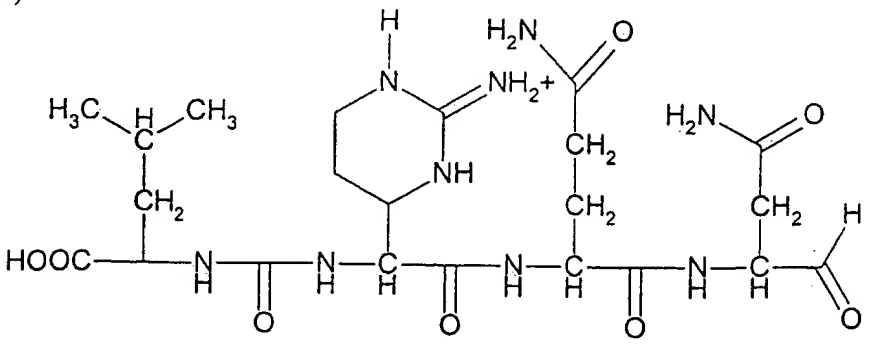


Figure 5 (page 1 of 6)

E-64

Structure: L-trans-epoxysuccinyl-leucylamide-(4-guanido)-butane or N-[N-(L-trans-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine

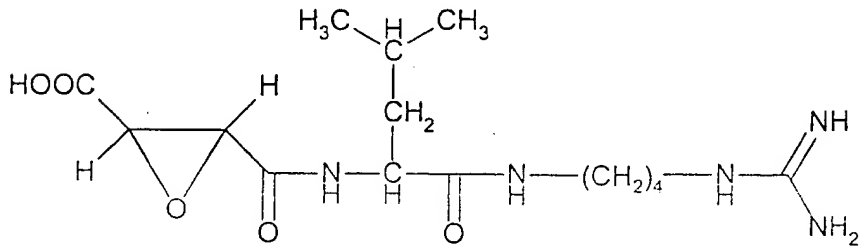


Figure 5 (page 2 of 6)

Leupeptin

Structure: Acetyl-leucyl-leucyl arginal

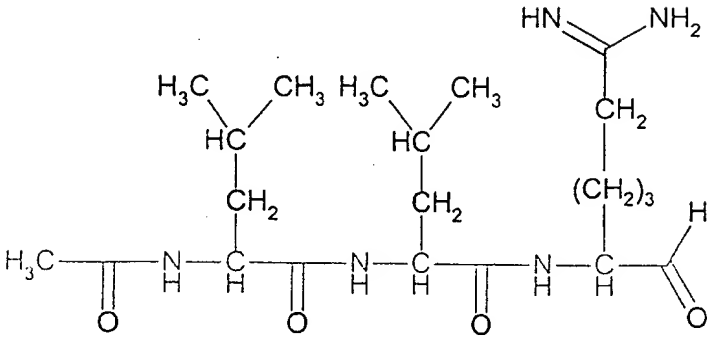


Figure 5 (page 3 of 6)

Antipain

Structure: [(S)-1-Carboxy-2-Phenyl]-carbamoyl-Arg-Val-arginal

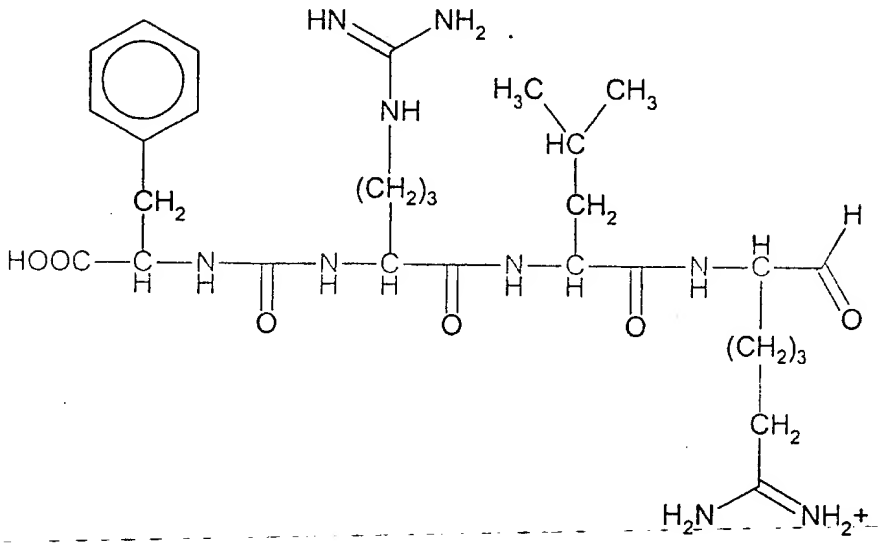


Figure 5 (page 4 of 6)

Elastinal

Structure: Leu-(Cap)-Gln-Ala-al, N-[(S)-1carboxy-isopentyl)-carbamoyl-alpha-(2-iminohexahydro-4(S)-pyrimidyl]-L-glycyl-L-glutaminyl-L-alaninal

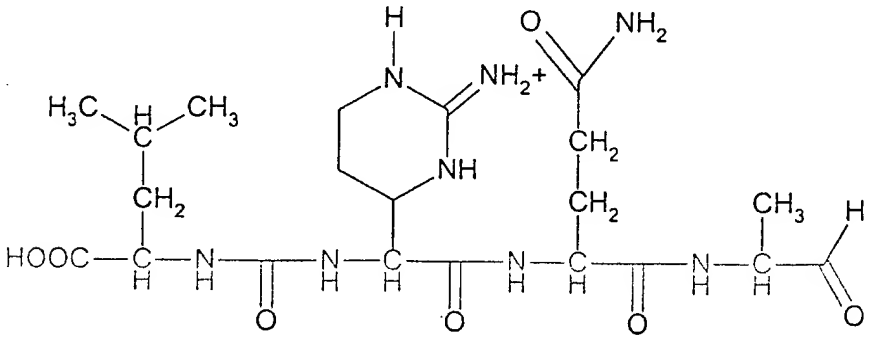


Figure 5 (page 5 of 6)

TLCK

Structure: Tosyl Lysyl ChloromethylKetone:
1-Chloro-3-tosylamido-7-amino-2-heptanone

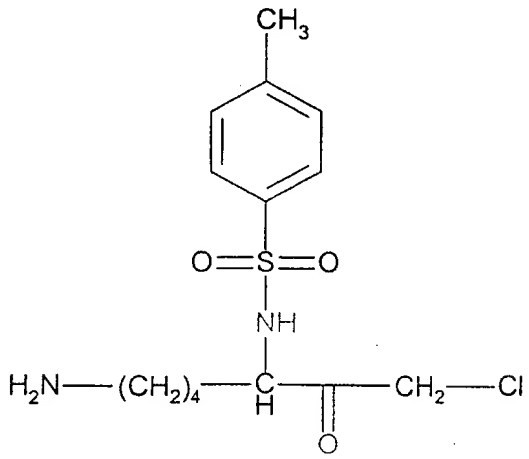


Figure 5 (page 6 of 6)

TPCK

Structure: Tosyl Phenylalanyl ChloromethylKetone:
1-Chloro-3-tosylamido-4-phenyl-2-butanone

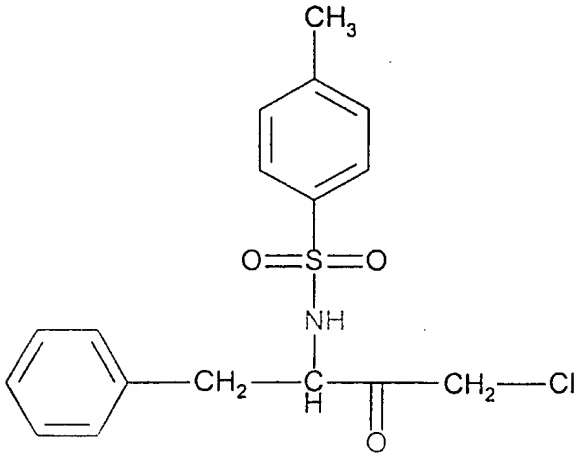
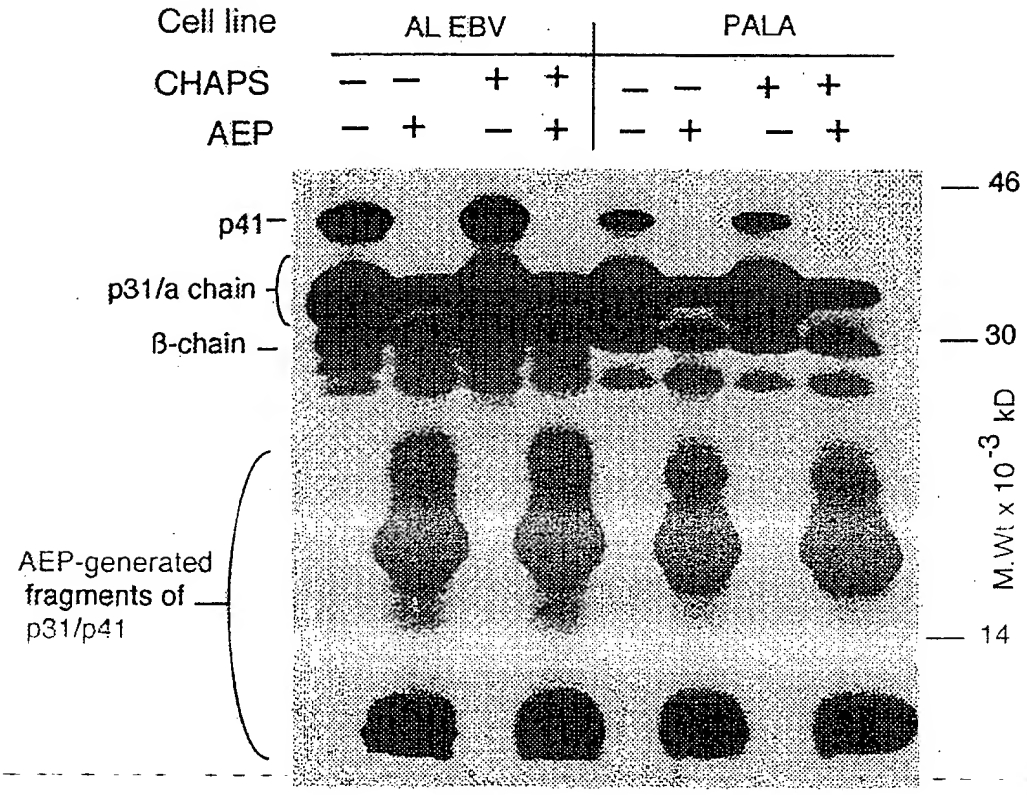


Fig. 6


Cell line
AL EBV
PALA
CHAPS
AEP
p41
p31/a chain
ß-chain
AEP-generated fragments of p31/p41
46
30
14
M.Wt x 10-3 kD

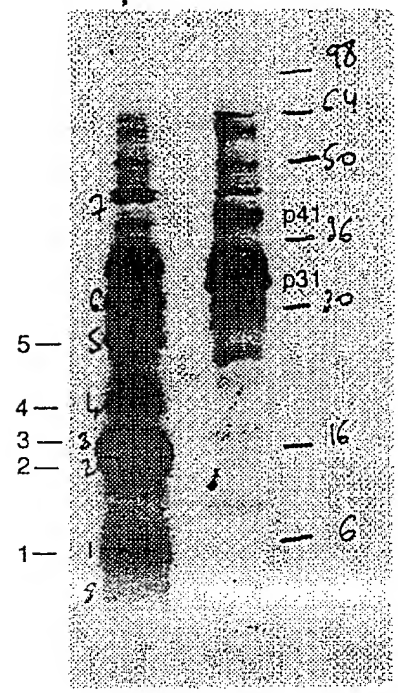
AEP processing of the invariant chain

Fig. 7

(a)

VIC γ1 precipitate

AEP + −



(b) N-terminal sequence of bands below

Band 1: N NEQLPM (Peak cycle 5/6)

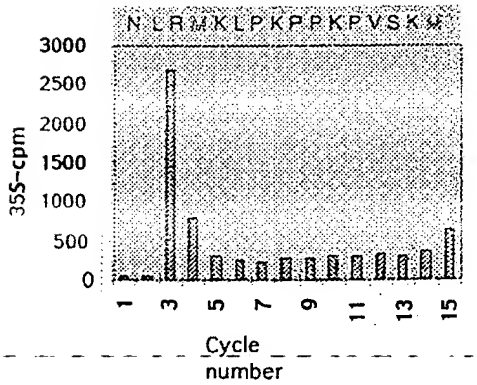
Band 2: N TMETI (Peak cycle 2)

Band 3: N LRMK (Peak cycle 3)

Band 4: N LRMK
N NEQLPM (Peaks at cycles 3 & 6)

Band 5: N LRMK

Example raw data from band 5



(c)

MDDQRDLISNNEQLPMLGRRPGAPESKCSRGALYTGFSILVTLLLAGQATTAYF

QQQGRLDKLTVTSQNLQLENLRMKLPKPPKPVSKMRMATPLLMQALPMGALPQG

QNATKYGNMTEDHVMHLLQNADPLKVYPPLKGSFPENLTHLKNTMETIDWKVFE

MHHWLLFEMSRHSLEQKPTDAPPKESLELEDPSSGLGVTKQDLGPVPM

SQ 17

Fig 8

Identified AEP cleavage sites

Tetanus toxin C fragment

RHIDN EEDID
YTPNN EIDSF
GNAFN NLDRI

Ribonuclease

NGQTN CYQSY
VACKN GQTNC

Ovalbumin

GTSVN VHSSL

Hen Egg Lysozyme
(preferred sites listed first)

GNGMN AWVAW
HGLDN YRGYS
ILQIN SRWWC
VSDGN GMNAW
RWWCN DGRTP
VAWRN RCKGT

Transferrin peptide
(622-642)

LFGSN VTDCS
DCSGN FCLFR

Fig. 9 (page 1 of 2)

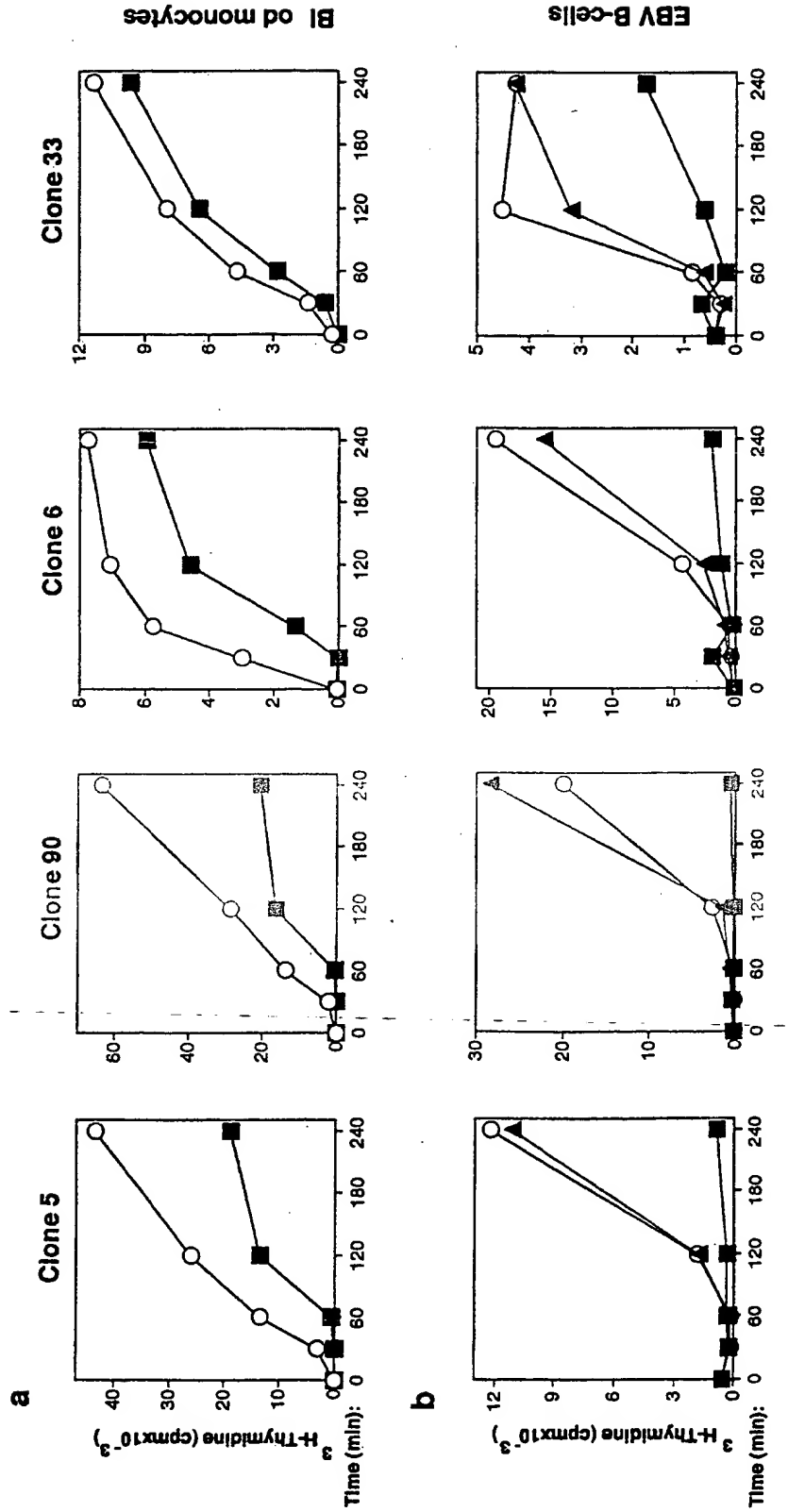


Fig 9 (page 2 of 2)